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(54) Title: METHOD TO PREDICT OR DIAGNOSE A GASTROINTESTINAL DISORDER OR DISEASE

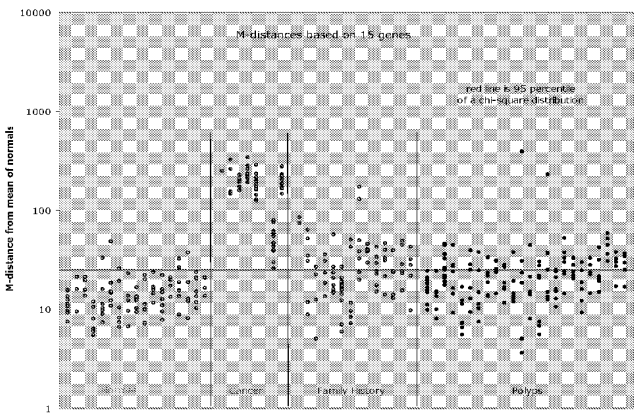


FIGURE 1A

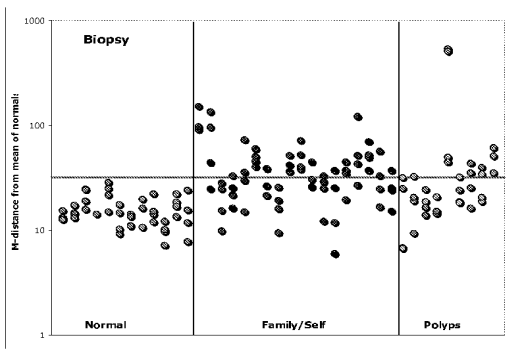


FIGURE 1B

(57) Abstract: The disclosure provides methods and compositions useful for identifying a subject's predisposition to a gastrointestinal disease or disorder.

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# METHOD TO PREDICT OR DIAGNOSE A GASTROINTESTINAL DISORDER OR DISEASE

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** The application claims priority under 35 U.S.C. §119 to U.S. Provisional Application Serial No. 60/941,195, filed May 31, 2007, the disclosure of which is incorporated herein by reference.

## TECHNICAL FIELD

**[0002]** The invention relates to predicting the probability that a subject has a predisposition to or has a gastrointestinal tract disease or disorder.

## BACKGROUND

**[0003]** Presently, there are no biological tests in clinical use to predict a subject's probability, propensity or presence of a gastrointestinal disorder based upon gene expression profiling.

## SUMMARY

**[0004]** The disclosure provides a method of diagnosing a cancer or inflammatory disease or disorder in the gastrointestinal tract of an asymptomatic subject. The method comprises (i) collecting mucosal epithelial cells from the buccal area (e.g., the oral cavity or mouth including the tongue, sublingual, gums and inner cheek) of the subject; (ii) measuring the expression level of at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more polynucleotides selected from the group consisting of CXCR2, OPN, COX1, PPARα, COX2, IL8, P21, c-Myc,CD44, and PPARδ. The polynucleotide can comprise a sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, and 43 and naturally occurring variants thereof. The polynucleotides are detected in the collected cells and the expression or amount of the polynucleotide compared to the level of the same polynucleotides in a normal control, wherein a change in the expression level is indicative of cancer or non-colorectal inflammatory disease or disorder. In one embodiment, at least 10 polynucleotides comprising a panel are detected and compared.

**[0005]** The disclosure also provides a method of diagnosing a non-colorectal cancer inflammatory disease or disorder in the gastrointestinal tract of an asymptomatic subject believed to have an inflammatory disease or disorder of their gastrointestinal

tract. The method comprises (i) swabbing the buccal or rectal area of the subject to collect epithelial cells; (ii) measuring the expression level of at least two polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43 in the collected cells; (iii) comparing the expression level of the at least two polynucleotides to the level of the same polynucleotide in a normal control, wherein a change in the expression level is indicative of non-colorectal cancer inflammatory disease or disorder.

**[0006]** The disclosure further provides a method of diagnosing an inflammatory disease or disorder of the gastrointestinal tract. The method comprises (a) contacting a buccal or rectal sample from a subject with at least one probe comprising at least 8 contiguous nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43; and (b) quantifying the amount of a polynucleotide molecule that hybridizes to the at least one probe, wherein an increase in the amount of polynucleotide relative to a normal control is indicative of the subject having a gastrointestinal disease or disorder, and wherein the subject has no familial or self history of a gastrointestinal disease or disorder.

**[0007]** The disclosure provides a method of screening a subject for the risk of developing a cancer or a gastrointestinal disease or disorder, comprising: (a) contacting a buccal or rectal sample from the subject with at least one probe comprising at least 8 contiguous nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43; and (b) quantifying the amount of a polynucleotide molecule that hybridizes to the at least one probe, wherein an increase in the amount of polynucleotide relative to a control is indicative of the subject having a risk of developing a cancer or a gastrointestinal disease or disorder, and wherein the subject has no familial or self history of a gastrointestinal disease or disorder.

**[0008]** A method of diagnosing Crohn's disease in a subject believed to have Crohn's disease is provided by the disclosure. The method comprises (i) collecting mucosal epithelial cells from

the buccal or rectal area of the subject using a swab; (ii) measuring the expression level of at least two polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, or 5; (iii) comparing the expression level of the at least two polynucleotides to the level of the same polynucleotide in a normal control, wherein an increase in the expression level compared to the normal control is indicative Crohn's disease.

**[0009]** The disclosure also provides a method of diagnosing Barrett's disease in a subject believed to have Barrett's disease, comprising: (i) collecting mucosal epithelial cells from the buccal or rectal area of the subject using a swab; (ii) measuring the expression level of at least two polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, or 5; (iii) comparing the expression level of the at least two polynucleotides to the level of the same polynucleotide in a normal control, wherein an increase in the expression level compared to the normal control is indicative Barrett's disease.

**[0010]** Also provided is an oligonucleotide/DNA chip comprising a panel of biomarkers of at least 8 contiguous nucleotides from a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, 43 or any combination thereof. In a method of the disclosure a sample comprising polynucleotides obtained from the buccal area (*e.g.*, by swab) is contacted with the oligonucleotide/DNA chip and hydridizaiton between polynucleotides in the sample and a control are quantitated.

**[0011]** The disclosure also provides a method comprising: (a) providing a sample obtained from a buccal swab, the sample comprising polypeptides obtained from a subject; (b) contacting the sample with at least one probe that specifically binds to a polypeptide consisting essentially of a sequence as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, or 44; and (c) determining if the sample comprises the polypeptide, wherein an increase or decrease in a panel of the polypeptides relative to a normal control is indicative of the subject having or at risk of having a gastrointestinal disease or disorder.

**[0012]** Various kits are also provided by the disclosure for carrying out any of the methods described herein. For example, a kit can comprise an oligonucleotide probe or primer pair for detecting a polynucleotide comprising a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43. In yet another embodiment, the disclosure provides a kit comprising an agent the specifically detects a polypeptide comprising a sequence as set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42 or 44.

**[0013]** The details of one or more embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

## BRIEF DESCRIPTION OF THE FIGURES

**[0014]** Figure 1A-C shows the Mahalanobis distance for biopsy samples, taken from (left to right), controls, resected colon cancer, individuals with family history, and individuals with polyps (67 subject and 15 genes), (B) shows the same analysis carried out on a second patient pool, one including individuals with no polyps or family/self history (Control), individuals with family history, individuals with polyps, and (C) shows the same analysis carried out on rectal smear samples taken from the same individuals.

**[0015]** Figure 2A and B shows swab data. (A) shows a 90 patient study of gene expression values for 16 genes from each subject obtained by rectal swab, controls tend to fall below the 95% chi-square distribution line. A tendency of subjects with cancer to fall above the like can be seen at the far right. (B) shows the 95% chi-square distribution of gene analysis from buccal swabs of 21 controls and 8 cancer subjects.

## DETAILED DESCRIPTION

**[0016]** As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polynucleotide" includes a plurality of such polynucleotides

and reference to "the variant" includes reference to one or more variants known to those skilled in the art, and so forth.

**[0017]** Also, the use of "or" means "and/or" unless stated otherwise. Similarly, "comprise," "comprises," "comprising" "include," "includes," and "including" are interchangeable and not intended to be limiting.

**[0018]** It is to be further understood that where descriptions of various embodiments use the term "comprising," those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language "consisting essentially of" or "consisting of."

**[0019]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

**[0020]** The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

**[0021]** Outpatient clinical diagnostics are useful to reduce costs of unnecessary, often invasive or painful, procedures. As a screening tool, colonoscopy is considered too expensive, both to the patients and to the insurance carriers, and carries with it a small percentage of risks and complications. Barium enema and CT colonography (or virtual colonoscopy), like colonoscopy, will provide for a complete colon examination, but small polyps or even small cancers can be missed. The cost is high, and higher still if a polyp or cancer or even a suggestion of a polyp or cancer will be interpreted by the radiologists, requiring the additional procedure of colonoscopy for confirmation. The barium enema, the CT colonography and the colonoscopy procedures all require the patients to have a thorough mechanical bowel preparation the day before. The diagnostic tests and compositions described herein are

useful to identify, diagnose, and prognose subjects that should be followed or treated for gastrointestinal diseases and disorders including the development of polyps, cancerous lesions or other non-cancerous inflammatory diseases.

**[0022]** In some instances a subject may not have access or know their familial history. In such instances, the diagnostics of the disclosure can be used to determine if they have a predisposition to a gastrointestinal disease or disorder based upon a FHSH biomarker panel. In other aspects, where a subject is identified as having a FHSH GI disease or disorder, the subject may be monitored for changes in biomarker expression indicative of cancer lesions or polyps based upon a cancer biomarker panel. Where a biomarker panel associated with colorectal cancer is present the subject may be monitored by, for example, by colonoscopy for early detection and removal of polyps or cancerous lesions. One advantage of the biomarker panels provided herein, is that the panel may be detected by swab collection (e.g., swab of the buccal or rectal area of 5-10 cm). Such procedures may be performed in an outpatient setting. As indicated above, statistics indicate that early detection and removal of cancerous lesion and polyps reduce mobidity and mortality of subjects.

**[0023]** An adenoma, colon adenoma, flat adenoma and polyp are used herein to describe any precancerous neoplasia of the colon. Precancerous colon neoplasias are referred to as adenomas or adenomatous polyps. Adenomas are typically small mushroom-like or wart-like growths on the lining of the colon and do not invade into the wall of the colon. Adenomas may be visualized through a device such as a colonoscope or flexible sigmoidoscope. Several studies have shown that patients who undergo screening for and removal of adenomas have a decreased rate of mortality from colon cancer. For this and other reasons, it is generally accepted that adenomas are an obligate precursor for the vast majority of colon cancers. When a colon neoplasia invades into the basement membrane of the colon, it is considered a colon cancer. The most widely used staging systems generally use at least one of the following characteristics for staging: the extent of tumor penetration into the colon wall, with greater penetration generally correlating with a more

dangerous tumor; the extent of invasion of the tumor through the colon wall and into other neighboring tissues, with greater invasion generally correlating with a more dangerous tumor; the extent of invasion of the tumor into the regional lymph nodes, with greater invasion generally correlating with a more dangerous tumor; and the extent of metastatic invasion into more distant tissues, such as the liver, with greater metastatic invasion generally correlating with a more dangerous disease state.

**[0024]** An allele refers to a particular form of a genetic locus, distinguished from other forms by its particular nucleotide sequence, or one of the alternative polymorphisms found at a polymorphic site.

**[0025]** A biological sample refers to a sample obtained from a subject wherein the sample comprises cells, or can be cell free. The biological sample can be blood, sputum, saliva, tissue, stool, urine, serum cerebrospinal, or the like. Where the sample is a tissue, the tissue sample can be obtained by biopsy. Biopsy samples can be obtained from the gastrointestinal tract (*e.g.*, from a segment of colon between the cecum and the hepatic flexure were classified as ascending colon samples; those from the segment of colon between the hepatic flexure and the splenic flexure as transverse colon samples; those from the segment of colon below the splenic flexure as descending colon; those from the winding segment of colon below the descending colon were classified as rectosigmoid colon samples (approximately 5-25 cm from rectum)). The biological sample can be obtained non-invasively (*e.g.*, by swab). The swab, for example, can be obtained from the mouth or rectum. In one embodiment, the swab is obtained from the buccal area, such as the cheek or throat, of a subject. A minimally invasive method, such as a swab, or a non-invasive sampling method, such as a stool sample can be obtained and the swab or a preparation thereof used in the methods of the disclosure. A biopsy will tend to have a more heterogenous mixture of cell-types (e.g., epithelial, stromal and endothelial cells) compared to a swab sample, which has a higher percentage of cell types on the colorectal surface (e.g., epithelial and inflammatory cells).

**[0026]** A biomarker refers to a detectable biological entity associated with a particular phenotype or risk of developing a particular phenotype. The biological entity can be a polypeptide or polynucleotide. A biomarker to be detected is referred to as a target. For example, a target polynucleotide refers to a biomarker comprising a polynucleotide (*e.g.*, an mRNA or cDNA) that is to be detected. In another example, a target polypeptide refers to a protein expressed (*i.e.*, transcribed and translated) that is to be detected. A biomarker, as defined by the National Institutes of Health (NIH), refers to a molecular indicator of a specific biological property; a biochemical feature or facet that can be used to measure the progress of disease or the effects of treatment. A panel of biomarkers is a selection of at least two biomarkers. Biomarkers may be from a variety of classes of molecules. In principle, the larger the number of genes used, the more sensitive the analysis will be. However, as the panel increases in size, the analysis becomes more complex and time-consuming. The panel can comprise from 2 to sixteen or more genes or biomarkers. In one aspect, the panel comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or more genes or biomarkers. The results suggest for individuals with cancer, three or four genes, such as COX-2, IL-8 and CD44, can suffice. However, for individuals with polyps or with history of cancer fine-tuning the analysis by adding to or otherwise modifying the gene panel increases specificity.

**[0027]** The term "colon" as used herein is intended to encompass the right colon (including the cecum), the transverse colon, the left colon, and the rectum.

**[0028]** A control subject refers to individuals with no polyps and no family or self history of cancer or known upper GI problem. Subjects with either a family history of any cancer or personal history of any cancer, and with no polyps during a current colonoscopy are referred to as FHSH subjects. Subjects with polyps and with or without family or self history of any cancer are referred to as polyps subjects and comprise a FHSH subject's biomarker panel. Subjects with colon cancer are referred to as cancer subjects and comprise a cancer subject's biomarker panel.

**[0029]** A fecal occult blood test (FOBT) is a test used to check for hidden blood in the stool. Sometimes cancers or polyps can bleed, and FOBT is used to detect small amounts of bleeding. In addition, screening tests (such as a rectal examination, proctoscopy, and colonoscopy) may be done regularly in patients who are at high risk of colon cancer or who have a positive FOBT and/or biomarker results. The proctoscopy examination finds about half of all colon and rectal cancers. After treatment, a blood test and x-rays may be done to screen for recurrence.

**[0030]** Colorectal cancer, also referred to as colon cancer or large bowel cancer, includes cancerous growths in the colon, rectum and appendix. Many colorectal cancers arise from adenomatous polyps in the colon. These growths are usually benign, but some may develop into cancer over time. The majority of the time, the diagnosis of localized colon cancer is through colonoscopy. Therapy is usually through surgery, which in many cases is followed by chemotherapy. Polyps of the colon, particularly adenomatous polyps, are a risk factor for colon cancer. The removal of colon polyps at the time of colonoscopy reduces the subsequent risk of colon cancer. Individuals who have previously been diagnosed and treated for colon cancer are at risk for developing colon cancer in the future. Women who have had cancer of the ovary, uterus, or breast are at higher risk of developing colorectal cancer. Family history of colon cancer, especially in a close relative before the age of 55 or multiple relatives, increases the risk of cancer in a subject.

**[0031]** Gastrointestinal inflammation refers to inflammation of a mucosal layer of the gastrointestinal tract, and encompasses acute and chronic inflammatory conditions. Acute inflammation is generally characterized by a short time of onset and infiltration or influx of neutrophils. Chronic inflammation is generally characterized by a relatively longer period of onset and infiltration or influx of mononuclear cells. Chronic inflammation can also be characterized by periods of spontaneous remission and spontaneous occurrence. The mucosal layer of the gastrointestinal tract includes mucosa of the bowel (including the small intestine and large intestine), rectum, stomach (gastric) lining, oral

cavity, and the like. Examples of chronic gastrointestinal inflammation include inflammatory bowel disease (IBD), colitis induced by environmental insults (*e.g.*, gastrointestinal inflammation (*e.g.*, colitis) caused by or associated with (*e.g.*, as a side effect) a therapeutic regimen, such as administration of chemotherapy, radiation therapy, and the like), colitis in conditions such as chronic granulomatous disease (Schappi *et al.* Arch Dis Child. 2001 Feb;84(2):147-151), celiac disease, celiac sprue (a heritable disease in which the intestinal lining is inflamed in response to the ingestion of a protein known as gluten), food allergies, gastritis, infectious gastritis or enterocolitis (*e.g.*, Helicobacter pylori-infected chronic active gastritis) and other forms of gastrointestinal inflammation caused by an infectious agent, and other like conditions.

**[0032]** As used herein, "inflammatory bowel disease" or "IBD" refers to any of a variety of diseases characterized by inflammation of all or part of the intestines. Examples of inflammatory bowel disease include, but are not limited to, Crohn's disease, Barrett's disease and ulcerative colitis. Reference to IBD throughout the specification is often referred to in the specification as exemplary of gastrointestinal inflammatory conditions, and is not meant to be limiting. The term IBD includes pseudomembranous colitis, hemorrhagic colitis, hemolytic-uremic syndrome colitis, collagenous colitis, ischemic colitis, radiation colitis, drug and chemically induced colitis, diversion colitis, ulcerative colitis, irritable bowel syndrome, irritable colon syndrome, Barrett's disease and Crohn's disease; and within Crohn's disease all the subtypes including active, refractory, and fistulizing and Crohn's disease.

**[0033]** A non-colorectal cancer inflammatory disease or disorder of the gastrointestinal tract refers to an inflammation of the gastrointestinal tract in the absence of a cancerous lesion, tumor or lesion. A non-colorectal cancer inflammatory disease or disorder of the gastrointestinal tract includes inflammatory bowel disease.

**[0034]** A gene refers to a segment of genomic DNA that contains the coding sequence for a protein, wherein the segment may include

promoters, exons, introns, and other untranslated regions that control expression.

**[0035]** A genotype is an unphased 5' to 3' sequence of nucleotide pair(s) found at a set of one or more polymorphic sites in a locus on a pair of homologous chromosomes in an individual. As used herein, genotype includes a full-genotype and/or a sub-genotype.

**[0036]** Genotyping is a process for determining a genotype of an individual.

**[0037]** A haplotype is a 5' to 3' sequence of nucleotides found at a set of one or more polymorphic sites in a locus on a single chromosome from a single individual.

**[0038]** Haplotype pair is two haplotypes found for a locus in a single individual.

**[0039]** Haplotyping is the process for determining one or more haplotypes in an individual and includes use of family pedigrees, molecular techniques and/or statistical inference.

**[0040]** A genetic locus refers to a location on a chromosome or DNA molecule corresponding to a gene or a physical or phenotypic feature, where physical features include polymorphic sites.

**[0041]** Polymorphic site (PS) is a position on a chromosome or DNA molecule at which at least two alternative sequences are found in a population.

**[0042]** A polymorphism refers to the sequence variation observed in an individual at a polymorphic site. Polymorphisms include nucleotide substitutions, insertions, deletions and microsatellites and may, but need not, result in detectable differences in gene expression or protein function. A single nucleotide polymorphism (SNP) is a single change in the nucleotide variation at a polymorphic site.

**[0043]** An oligonucleotide probe or a primer refers to a nucleic acid molecule of between 8 and 2000 nucleotides in length, or is specified to be about 6 and 1000 nucleotides in length. More particularly, the length of these oligonucleotides can range from about 8, 10, 15, 20, or 30 to 100 nucleotides, but will typically be about 10 to 50 (*e.g.*, 15 to 30 nucleotides). The appropriate length for oligonucleotides in assays of the disclosure under a

particular set of conditions may be empirically determined by one of skill in the art.

**[0044]** Oligonucleotide primers and probes can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences and direct chemical synthesis. The oligonucleotide primers and probes can contain conventional nucleotides, as well as any of a variety of analogs. For example, the term "nucleotide", as used herein, refers to a compound comprising a nucleotide base linked to the C-1' carbon of a sugar, such as ribose, arabinose, xylose, and pyranose, and sugar analogs thereof. The term nucleotide also encompasses nucleotide analogs. The sugar may be substituted or unsubstituted. Substituted ribose sugars include, but are not limited to, those riboses in which one or more of the carbon atoms, for example the 2'-carbon atom, is substituted with one or more of the same or different Cl, F, --R, --OR, $--NR_2$ or halogen groups, where each R is independently H, $C_1$-$C_6$ alkyl or $C_5$-$C_{14}$ aryl. Exemplary riboses include, but are not limited to, 2'-($C_1$-$C_6$)alkoxyribose, 2'-($C_5$-$C_{14}$)aryloxyribose, 2',3'-didehydroribose, 2'-deoxy-3'-haloribose, 2'-deoxy-3'-fluororibose, 2'-deoxy-3'-chlororibose, 2'-deoxy-3'-aminoribose, 2'-deoxy-3'-($C_1$-$C_6$)alkylribose, 2'-deoxy-3'-($C_1$-$C_6$)alkoxyribose and 2'-deoxy-3'-($C_5$-$C_{14}$)aryloxyribose, ribose, 2'-deoxyribose, 2',3'-dideoxyribose, 2'-haloribose, 2'-fluororibose, 2'-chlororibose, and 2'-alkylribose, *e.g.*, 2'-O-methyl, 4'-α-anomeric nucleotides, 1'-α-anomeric nucleotides, 2'-4'- and 3'-4'-linked and other "locked" or "LNA", bicyclic sugar modifications (see, *e.g.*, PCT published application nos. WO 98/22489, WO 98/39352;, and WO 99/14226). Exemplary LNA sugar analogs within a polynucleotide include, but are not limited to, the structures: where B is any nucleotide base.

**[0045]** Modifications at the 2'- or 3'-position of ribose include, but are not limited to, hydrogen, hydroxy, methoxy, ethoxy, allyloxy, isopropoxy, butoxy, isobutoxy, methoxyethyl, alkoxy, phenoxy, azido, amino, alkylamino, fluoro, chloro and bromo. Nucleotides include, but are not limited to, the natural D optical isomer, as well as the L optical isomer forms (see, *e.g.*, Garbesi (1993) Nucl. Acids Res. 21:4159-65; Fujimori (1990) J. Amer. Chem. Soc. 112:7435; Urata, (1993) Nucleic Acids Symposium

Ser. No. 29:69-70). When the nucleotide base is purine, *e.g.* A or G, the ribose sugar is attached to the $N_9$-position of the nucleotide base. When the nucleotide base is pyrimidine, *e.g.* C, T or U, the pentose sugar is attached to the $N_1$-position of the nucleotide base, except for pseudouridines, in which the pentose sugar is attached to the $C_5$ position of the uracil nucleotide base (see, *e.g.*, Kornberg and Baker, (1992) DNA Replication, 2nd Ed., Freeman, San Francisco, Calif.). The 3' end of the probe can be functionalized with a capture or detectable label to assist in detection of a target polynucleotide or of a polymorphism.

**[0046]** Any of the oligonucleotides or nucleic acids of the disclosure can be labeled by incorporating a detectable label measurable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, such labels can comprise radioactive substances (*e.g.*, $^{32}P$, $^{35}S$, $^{3}H$, $^{125}I$), fluorescent dyes (*e.g.*, 5-bromodesoxyuridin, fluorescein, acetylaminofluorene, digoxigenin), biotin, nanoparticles, and the like. Such oligonucleotides are typically labeled at their 3' and 5' ends.

**[0047]** A probe refers to a molecule which can detectably distinguish changes in gene expression or can distinguish between target molecules differing in structure. Detection can be accomplished in a variety of different ways depending on the type of probe used and the type of target molecule. Thus, for example, detection may be based on discrimination of activity levels of the target molecule, but typically is based on detection of specific binding. Examples of such specific binding include antibody binding and nucleic acid probe hybridization. Thus, for example, probes can include enzyme substrates, antibodies and antibody fragments, and nucleic acid hybridization probes (including primers useful for polynucleotide amplification and/or detection). Thus, in one embodiment, the detection of the presence or absence of the at least one target polynucleotide involves contacting a biological sample with a probe, typically an oligonucleotide probe, where the probe hybridizes with a form of a target polynucleotide in the biological sample containing a complementary sequence, where the hybridization is carried out under selective hybridization

conditions. Such an oligonucleotide probe can include one or more nucleic acid analogs, labels or other substituents or moieties so long as the base-pairing function is retained.

**[0048]** A reference or control population refers to a group of subjects or individuals who are predicted to be representative of the genetic variation found in the general population having a particular genotype or expression profile. Typically, the reference population represents the genetic variation in the population at a certainty level of at least 85%, typically at least 90%, least 95% and but commonly at least 99%. The reference or control population can include subjects who individually have not demonstrated any gastrointestinal disease or disorder and can include individuals whose family line does not or has not demonstrated any gastrointestinal diseases or disorders.

**[0049]** A subject comprises an individual (*e.g.*, a mammalian subject or human) whose gene expression profile, genotypes or haplotypes or response to treatment or disease state are to be determined.

**[0050]** The disclosure provides a number of biomarkers useful for predicting a subject's predisposition or the existence of a gastrointestinal disease or disorder. The biomarkers identified herein can be used in combination with additional predictive tests including, but not limited to, additional SNPs, mutations, and clinical tests.

**[0051]** One embodiment of what is disclosed is the measurement of at least one or a panel of biomarkers with the selectivity and sensitivity required for managing and diagnosing subjects that have or may have a predisposition to a gastrointestinal disease or disorder. Table 1 provides a list of polynucleotide biomarkers useful in the methods and compositions of the disclosure (each of the sequences associated with the Entrez Accession Nos. set forth in Table 1 are incorporated herein by reference).

TABLE 1

| **SEQ ID NO: polynucleotide and polypeptide** | **NCBI Entrez Database** | **Name** | **Abbreviation** |
|---|---|---|---|
| 1 and 2 | XM_031289 | Interleukin-8 | IL8 |
| 3 and 4 | NM_000389 | cyclin-dependent kinase inhibitor | P21 |

| | | | |
|---|---|---|---|
| | | 1A (p21, Cip1) | |
| 5 and 6 | XM_030326 | CD44 antigen | CD44 |
| 7 and 8 | M94582 | Interleukin 8 receptor B | CXCR2 |
| 9 and 10 | X54489 | Melanoma growth stimulatory activity | Gro-alpha |
| 11 and 12 | NM_002090 | Chemokine (C-X-C motif) ligand3 | Gro-gamma |
| 13 and 14 | XM_003059 | Peroxisome proliferative activated receptor, gamma | PPAR-gamma |
| 15 and 16 | NM_006238 | Peroxisome proliferative activated receptor, delta | PPAR-delta |
| 17 and 18 | AX057136 | c-Myc | c-Myc |
| 19 and 20 | XM_032429 | Secreted phosphoprotein 1 | SPP1 (OPN) |
| 21 and 22 | XM_044882 | Prostaglandin-endoperoxide synthase 1 | COX-1 |
| 23 and 24 | XM_051900 | Prostaglandin-endoperoxide synthase 2 | COX-2 |
| 25 and 26 | NM_005036 | Peroxisome proliferative activated receptor, alpha | PPAR-alpha |
| 27 and 28 | NM_000757 | Macrophage colony stimulating factor 1 | MCSF-1 |
| 29 and 30 | M64349 | Cyclin-D | Cyc-D |
| 31 and 32 | NM_000331 | Serum amyloid A1 | SAA1 |
| 33 and 34 | NM_002131 | Homo sapiens high mobility group AT-hook 1 (HMGA1) | HMGA1 |
| 35 and 36 | X54942 X55506 | CKSHS2 | CKSHS2 |
| 37 and 38 | U22055 | Human 100 kDa coactivator | p100 activator |
| 39 and 40 | NM_005555 | Homo sapiens keratin 6B | LCN2 |
| 41 and 42 | BC021998 | Homo sapiens cyclin-dependent kinase inhibitor 2A | hCDK2a |
| 43 and 44 | NM_058195 | Homo sapiens cyclin-dependent kinase inhibitor 2A | hCDK2a alt. |

**[0052]** Homologs and naturally occurring variants (*e.g.*, polymorphisms) of any of the foregoing polynucleotides identified in Table 1 are encompassed by the disclosure. Identification of such naturally occurring polymorphisms are routinely identified or are known in the art. For example, polymorphisms of IL-8 and CXCR2

include SNP -251, -353/+1530, -353/+3331, and +1530/+3331 of IL-8 and +785/+1208 of CXCR2. Others include IL1B -31 SNP (C to T), IL10 -819 T/T. RS numbers include rs1143627 (IL1B), rs2243250 and rs1143634 (IL4), rs1801282 (PPAR-gamma), rs4073 (IL8), rs1800629 (TNF), and rs20417, rs5277, rs20432 and rs5275 (COX2).

**[0053]** In one aspect of the disclosure, expression levels of polynucleotides comprising biomarkers indicated in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 or 43 are used in the determination of a gastrointestinal disease or disorder or a predisposition to a gastrointestinal disease or disorder. Such analysis of polynucleotide expression levels is frequently referred to in the art as gene expression profiling. In gene expression profiling, levels of mRNA in a sample are measured as a indicator of a biological state, in this case, as an indicator of a colon cancer or gastrointestinal disease or disorder or a predisposition thereto. One of the most common methods for analyzing gene expression profiling is to create multiple copies from mRNA in a biological sample using a process known as reverse transcription. In the process of reverse transcription, the mRNA from the sample is used to create DNA copies of the corresponding mRNA. The copies made from mRNA are referred to as copy DNA, or cDNA. mRNA is somewhat unstable and subject degradation by RNAses. In one aspect, the RNA can be protected by using RNAse inhibitors and cocktails known in the art. Table 2 provides probes and primers useful to detecting a polynucleotide biomarker of the disclosure.

Table 2

| Sequence ID No./ID | Sequence | Name |
|---|---|---|
| 45. Forward Primer | agatattgca cgggagaata tacaaa | Interleukin 8 |
| 46. Reverse Primer | tcaattcctg aaattaaagt tcggata | |
| 47. Forward Primer | tctgcagagt tggaagcact cta | Prostaglandin-endoperoxide synthase 2 |
| 48. Reverse Primer | gccgaggctt ttctaccaga a | |
| 49. Forward Primer | catggcttga tcagcaagga | Interleukin 8 receptor B (CXCR2) |
| 50. Reverse Primer | tggaagtgtg ccctgaagaa g | |
| 51. Forward Primer | caaggagctg acttcggaac taa | Lipocalin 2 |
| 52. Reverse Primer | agggaagacg atgtggtttt ca | |
| 53. Forward Primer | gggacatgtg gagagcctac tc | Serum amyloid A1 |
| 54. Reverse Primer | catcatagtt cccccgagca t | |

| Sequence ID No./ID | Sequence | Name |
|---|---|---|
| 55. Forward Primer | aagcagcacc agcaagtgaa g | Macrophage colony stimulating factor 1 |
| 56. Reverse Primer | tcatggcctg tgtcagtcaa a | |
| 57. Forward Primer | acatgccagc cactgtgata g | Melanoma growth stimulatory activity |
| 58. Reverse Primer | ccctgccttc acaatgatct c | |
| 59. Forward Primer | ggaattcacc tcaagaacat cca | Chemokine (C-X-C motif) ligand 3 |
| 60. Reverse Primer | agtgtggcta tgacttcggt ttg | |
| 61. Forward Primer | cagccacaag cagtccagat ta | (OPN) Secreted phosphoprotein 1 |
| 62. Reverse Primer | cctgactatc aatcacatcg gaat | |
| 63. Forward Primer | ccaggtgctc cacatgacag t | Cyclin D |
| 64. Reverse Primer | aaacaaccaa caacaaggag aatg | |
| 65. Forward Primer | cgtctccaca catcagcaca a | c-Myc |
| 66. Reverse Primer | tcttggcagc aggatagtcc tt | |
| 67. Forward Primer | gcagaccagc atgacagatt tc | Cyclin-dependent kinase inhibitor (p21) |
| 68. Reverse Primer | gcggattagg gcttcctctt | |
| 69. Forward Primer | ggcaccagag gcagtaacca t | Cyclin-dependent kinase inhibitor 2A |
| 70. Reverse Primer | agcctctctg gttctttcaa tcg | |
| 71. Forward Primer | tggttcacat cccgcggct | Alternative reading frame p14 |
| 72. Reverse Primer | tggctcctca gtagcatcag | |
| 73. Forward Primer | tgaagttcaa tgcactggaa ctg | Peroxisome proliferation activated receptor, alpha |
| 74. Reverse Primer | caggacgatc tccacagcaa | |
| 75. Forward Primer | tggagtccac gagatcattt aca | Peroxisome proliferation activated receptor, gamma |
| 76. Reverse Primer | agccttggcc ctcggatat | |
| 77. Forward Primer | cactgagttc gccaagagca t | Peroxisome proliferation activated receptor, delta |
| 78. Reverse Primer | cacgccatac ttgagaaggg taa | |
| 79. Forward Primer | gctagtgatc aacagtggca atg | CD44 antigen |
| 80. Reverse Primer | gctggcctct ccgttgag | |
| 81. Forward Primer | tgttcggtgt ccagttccaa ta | Prostaglandin-endoperoxide synthase 1 |
| 82. Reverse Primer | tgccagtggt agagatggtt ga | |
| 83. Forward Primer | acaactccag gaaggaaacc aa | High-mobility group AT-hook1 isoform B |
| 84. Reverse Primer | cgaggactcc tgcgagatg | |
| 85. Forward Primer | tgaagaggag tggaggagac ttg | CKS1 protein homolog |
| 86. Reverse Primer | gaatatgtgg ttctggctca tgaa | |
| 87. Forward Primer | gagaaggagc gatctgctag ct | 100 kDa coactivator |
| 88. Reverse Primer | cacgtagaag tgcaggtcat cag | |

**[0054]** Methods known in the art can be used to quantitatively measure the amount of mRNA transcribed by cells present in a

sample. Examples of such methods include quantitative polymerase chain reaction (PCR), northern and southern blots. PCR allows for the detection and measurement of very low quantities of mRNA using an amplification process. Genes may either be up regulated or down regulated in any particular biological state, and hence mRNA levels shift accordingly.

**[0055]** In one embodiment, a method for gene expression profiling comprises measuring mRNA levels for biomarkers selected in a panel. Such a method can include the use of primers, probes, enzymes, and other reagents for the preparation, detection, and quantitation of mRNA (*e.g.*, by PCR, by Northern blot and the like). The primers listed in SEQ ID NOs: 45-88 are particularly suited for use in gene expression profiling using RT-PCR based on a polynucleotide biomarker. Although the disclosure provides particular primers and probes, those of skill in the art will readily recognize that additional probes and primers can be generated based upon the polynucleotide sequences provided by the disclosure. Referring to the primers and probes exemplified herein, a series of primers were designed using Primer Express Software (Applied Biosystems, Foster City, Calif.). The primers listed in SEQ ID NOs: 45-88 were designed, selected, and tested accordingly. In addition to the primers, reagents such as a dinucleotide triphosphate mixture having all four dinucleotide triphosphates (*e.g.*, dATP, dGTP, dCTP, and dTTP), a reverse transcriptase enzyme, and a thermostable DNA polymerase were used for RT-PCR. Additionally buffers, inhibitors and activators can also be used for the RT-PCR process. Once the cDNA has been sufficiently amplified to a specified end point, the cDNA sample can be prepared for detection and quantitation. Though a number of detection schemes are contemplated, as will be discussed in more detail below, one method contemplated for detection of polynucleotides is fluorescence spectroscopy, and therefore labels suited to fluorescence spectroscopy are desirable for labeling polynucleotides. One example of such a fluorescent label is SYBR Green, though numerous related fluorescent molecules are known including, without limitation, DAPI, Cy3, Cy3.5, Cy5, CyS.5, Cy7, umbelliferone, fluorescein, fluorescein isothiocyanate (FITC),

rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin.

**[0056]** In one embodiment of the disclosure, an oligonucleotide probe comprises a fragment of c-myc, CD44 antigen ("CD44"), cyclooxygenase 1 and 2("COX-1" and "COX-2"), cyclin D1, cyclin-dependent kinase inhibitor ("$p21^{cip/waf1}$"), interleukin 8 ("IL-8"), interleukin 8 receptor ("CXCR2"), osteopontin ("OPN"), melanoma growth stimulatory activity ("Groa/MGSA"), GRO3 oncogene ("Groγ"), macrophage colony stimulating factor 1 ("MCSF-1"), peroxisome proliferative activated receptor, alpha, delta and gamma ("PPAR-α, Δ and γ") and serum amyloid A1 ("SM 1") as set forth in Table 1.

**[0057]** Oligonucleotide probes and primers useful in the methods of the disclosure comprise at least 8 nucleotides of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, or 43 (including an oligonucleotide wherein T can be U) wherein the oligonucleotide specifically hybridizes to a polynucleotide sample from a subject comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 , 25, 27, 29, 31, 33, 35, 37, 39, 41 or 43.

**[0058]** Any of the oligonucleotide primers and probes of the disclosure can be immobilized on a solid support. Solid supports are known to those skilled in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, glass and the like. The solid support is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips and the like are all suitable examples. Suitable methods for immobilizing oligonucleotides on a solid phase include ionic, hydrophobic, covalent interactions and the like. The solid support can be chosen for its intrinsic ability to attract and immobilize the capture reagent. The oligonucleotide probes or primers of the disclosure can be attached to or immobilized on a solid support individually or in groups of about 2-10,000 distinct oligonucleotides of the disclosure to a single solid support.

**[0059]** A substrate comprising a plurality of oligonucleotide primers or probes of the disclosure may be used either for detecting or amplifying targeted sequences. The oligonucleotide probes and primers of the disclosure can be attached in contiguous regions or at random locations on the solid support. Alternatively the oligonucleotides of the disclosure may be attached in an ordered array wherein each oligonucleotide is attached to a distinct region of the solid support which does not overlap with the attachment site of any other oligonucleotide. Typically, such oligonucleotide arrays are "addressable" such that distinct locations are recorded and can be accessed as part of an assay procedure. The knowledge of the location of oligonucleotides on an array make "addressable" arrays useful in hybridization assays. For example, the oligonucleotide probes can be used in an oligonucleotide chip such as those marketed by Affymetrix and described in U.S. Pat. No. 5,143,854; PCT publications WO 90/15070 and 92/10092, the disclosures of which are incorporated herein by reference. These arrays can be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis.

**[0060]** The immobilization of arrays of oligonucleotides on solid supports has been rendered possible by the development of a technology generally referred to as "Very Large Scale Immobilized Polymer Synthesis" in which probes are immobilized in a high density array on a solid surface of a chip (see, *e.g.*, U.S. Patent Nos. 5,143,854; and 5,412,087 and in PCT Publications WO 90/15070, WO 92/10092 and WO 95/11995, each of which are incorporated herein by reference), which describe methods for forming oligonucleotide arrays through techniques such as light-directed synthesis techniques.

**[0061]** In another aspect, an array of oligonucleotides complementary to subsequences of the target gene is used to determine the identity of the target, measure its amount, and detect differences between the target and a reference wild-type sequence.

**[0062]** Hybridization techniques can also be used to identify the biomarkers and/or polymorphisms of the disclosure and thereby determine or predict a colorectal cancer or gastrointestinal inflammatory disease or disorder. In this aspect, expression profiles or polymorphism(s) are identified based upon the higher thermal stability of a perfectly matched probe compared to the mismatched probe. The hybridization reactions may be carried out in a solid support (*e.g.*, membrane or chip) format, in which, for example, the target nucleic acids are immobilized on nitrocellulose or nylon membranes and probed with oligonucleotide probes of the disclosure. Any of the known hybridization formats may be used, including Southern blots, slot blots, "reverse" dot blots, solution hybridization, solid support based sandwich hybridization, bead-based, silicon chip-based and microtiter well-based hybridization formats.

**[0063]** Hybridization of an oligonucleotide probe to a target polynucleotide may be performed with both entities in solution, or such hybridization may be performed when either the oligonucleotide or the target polynucleotide is covalently or noncovalently affixed to a solid support. Attachment may be mediated, for example, by antibody-antigen interactions, poly-L-Lys, streptavidin or avidin-biotin, salt bridges, hydrophobic interactions, chemical linkages, UV cross-linking baking, etc. Oligonucleotides may be synthesized directly on the solid support or attached to the solid support subsequent to synthesis. Solid-supports suitable for use in detection methods of the disclosure include substrates made of silicon, glass, plastic, paper and the like, which may be formed, for example, into wells (as in 96-well plates), slides, sheets, membranes, fibers, chips, dishes, and beads. The solid support may be treated, coated or derivatized to facilitate the immobilization of the allele-specific oligonucleotide or target nucleic acid.

**[0064]** In one aspect, a sandwich hybridization assay comprises separating the variant and/or wild-type target nucleic acid biomarker in a sample using a common capture oligonucleotide immobilized on a solid support and then contact with specific probes useful for detecting the variant and wild-type nucleic

acids. The oligonucleotide probes are typically tagged with a detectable label.

**[0065]** Hybridization assays based on oligonucleotide arrays rely on the differences in hybridization stability of short oligonucleotides to perfectly matched and mismatched target variants. Each DNA chip can contain thousands to millions of individual synthetic DNA probes arranged in a grid-like pattern and miniaturized to the size of a dime or smaller. Such a chip may comprise oligonucleotides representative of both a wild-type and variant sequences.

**[0066]** Oligonucleotides of the disclosure can be designed to specifically hybridize to a target region of a polynucleotide. As used herein, specific hybridization means the oligonucleotide forms an anti-parallel double-stranded structure with the target region under certain hybridizing conditions, while failing to form such a structure when incubated with a different target polynucleotide or another region in the polynucleotide or with a polynucleotide lacking the desired locus under the same hybridizing conditions. Typically, the oligonucleotide specifically hybridizes to the target region under conventional high stringency conditions.

**[0067]** A nucleic acid molecule such as an oligonucleotide or polynucleotide is said to be a "perfect" or "complete" complement of another nucleic acid molecule if every nucleotide of one of the molecules is complementary to the nucleotide at the corresponding position of the other molecule. A nucleic acid molecule is "substantially complementary" to another molecule if it hybridizes to that molecule with sufficient stability to remain in a duplex form under conventional low-stringency conditions. Conventional hybridization conditions are described, for example, in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and in Haymes *et al.*, Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, D.C. (1985). While perfectly complementary oligonucleotides are used in most assays for detecting target polynucleotides or polymorphisms, departures from complete complementarity are contemplated where such departures do not prevent the molecule from specifically hybridizing to the target

region. For example, an oligonucleotide primer may have a non-complementary fragment at its 5' or 3' end, with the remainder of the primer being complementary to the target region. Those of skill in the art are familiar with parameters that affect hybridization; such as temperature, probe or primer length and composition, buffer composition and salt concentration and can readily adjust these parameters to achieve specific hybridization of a nucleic acid to a target sequence.

**[0068]** A variety of hybridization conditions may be used in the disclosure, including high, moderate and low stringency conditions; see for example Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, *et al.*, hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point ($T_m$) for the specific sequence at a defined ionic strength and pH. The $T_m$ is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the polyadenylated mRNA target sequence at equilibrium (as the target sequences are present in excess, at $T_m$, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60° C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of helix destabilizing agents such as formamide. The hybridization conditions may also vary when a non-ionic backbone, *i.e.*, PNA is used, as is known in the art. In addition, cross-linking agents may be added after target binding to

cross-link, *i.e.*, covalently attach, the two strands of the hybridization complex.

**[0069]** Methods and compositions of the disclosure are useful for diagnosing or determining the risk of developing a colorectal cancer or gastrointestinal inflammatory disease or disorder. Such tests can be performed using DNA or RNA samples collected from blood, cells, tissue scrapings or other cellular materials, and can be performed by a variety of methods including, but not limited to, hybridization with biomarker-specific probes, enzymatic mutation detection, chemical cleavage of mismatches, mass spectrometry or DNA sequencing, including minisequencing. Diagnostic tests may involve a panel of one or more genetic markers (gene expression profiles), often on a solid support, or using PCR techniques, which enables the simultaneous determination of more than one variance in one or more genes or expression of one or more genes.

**[0070]** A target biomarker or region(s) thereof (*e.g.*, containing a polymorphism of interest) may be amplified using any oligonucleotide-directed amplification method including, but not limited to, polymerase chain reaction (PCR) (U.S. Pat. No. 4,965,188), ligase chain reaction (LCR) (Barany *et al.*, Proc. Natl. Acad. Sci. USA 88:189-93 (1991); WO 90/01069), and oligonucleotide ligation assay (OLA) (Landegren *et al.*, Science 241:1077-80 (1988)). Other known nucleic acid amplification procedures may be used to amplify the target region(s) including transcription-based amplification systems (U.S. Pat. No. 5,130,238; European Patent No. EP 329,822; U.S. Pat. No. 5,169,766; WO 89/06700) and isothermal methods (Walker *et al.*, Proc. Natl. Acad. Sci. USA 89:392-6 (1992)).

**[0071]** Ligase Chain Reaction (LCR) techniques can be used and are particularly useful for detection of polymorphic variants. LCR occurs only when the oligonucleotides are correctly base-paired. The Ligase Chain Reaction (LCR), which utilizes the thermostable Taq ligase for ligation amplification, is useful for interrogating loci of a gene (*e.g.*, comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41 or 43). A method of DNA amplification similar to PCR, LCR differs from PCR because it amplifies the probe molecule rather than producing

amplicon through polymerization of nucleotides. Two probes are used per each DNA strand and are ligated together to form a single probe. LCR uses both a DNA polymerase enzyme and a DNA ligase enzyme to drive the reaction. Like PCR, LCR requires a thermal cycler to drive the reaction and each cycle results in a doubling of the target nucleic acid molecule. LCR can have greater specificity than PCR. The elevated reaction temperatures permits the ligation reaction to be conducted with high stringency. Where a mismatch occurs, ligation cannot be accomplished. For example, a primer based upon a target gene or gene variant is synthesized in two fragments and annealed to the template with possible mutation at the boundary of the two primer fragments (*i.e.*, the underlined nucleotide above would be found at the 5' or 3' end of the oligonucleotide). A ligase ligates the two primers if they match exactly to the template sequence.

**[0072]** In one embodiment, the two hybridization probes are designed each with a target specific portion. The first hybridization probe is designed to be substantially complementary to a first target domain of a target polynucleotide (*e.g.*, a polynucleotide fragment) and the second hybridization probe is substantially complementary to a second target domain of a target polynucleotide (*e.g.*, a polynucleotide fragment). In general, each target specific sequence of a hybridization probe is at least about 5 nucleotides long, with sequences of about 15 to 30 being typical and 20 being especially common. In one embodiment, the first and second target domains are directly adjacent, *e.g.*, they have no intervening nucleotides. In this embodiment, at least a first hybridization probe is hybridized to the first target domain and a second hybridization probe is hybridized to the second target domain. If perfect complementarity exists at the junction, a ligation structure is formed such that the two probes can be ligated together to form a ligated probe. If this complementarity does not exist (due to mismatch based upon a variant), no ligation structure is formed and the probes are not ligated together to an appreciable degree. This may be done using heat cycling, to allow the ligated probe to be denatured off the target polynucleotide such that it may serve as a template for further reactions. The

method may also be done using three hybridization probes or hybridization probes that are separated by one or more nucleotides, if dNTPs and a polymerase are added (this is sometimes referred to as "Genetic Bit" analysis).

**[0073]** Analysis of point mutations (*e.g.*, polymorphic variants) in DNA can also be carried out by using the polymerase chain reaction (PCR) and variations thereof. Mismatches can be detected by competitive oligonucleotide priming under hybridization conditions where binding of the perfectly matched primer is favored. In the amplification refractory mutation system technique (ARMS), primers are designed to have perfect matches or mismatches with target sequences either internal or at the 3' residue (Newton *et al.*, Nucl. Acids. Res. 17:2503-2516 (1989)). Under appropriate conditions, only the perfectly annealed oligonucleotide functions as a primer for the PCR reaction, thus providing a method of discrimination between normal and variant sequences.

**[0074]** Single nucleotide primer-guided extension assays can also be used, where the specific incorporation of the correct base is provided by the fidelity of a DNA polymerase. Detecting the nucleotide or nucleotide pair at a polymorphic site of interest may also be determined using a mismatch detection technique including, but not limited to, the RNase protection method using riboprobes (Winter *et al.*, Proc. Natl. Acad. Sci. USA 82:7575 (1985); Meyers *et al.*, Science 230:1242 (1985)) and proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, Ann. Rev. Genet. 25:229-53 (1991)). Alternatively, variant alleles can be identified by single strand conformation polymorphism (SSCP) analysis (Orita *et al.*, Genomics 5:874-9 (1989); Humphries *et al.*, in MOLECULAR DIAGNOSIS OF GENETIC DISEASES, Elles, ed., pp. 321-340, 1996) or denaturing gradient gel electrophoresis (DGGE) (Wartell *et al.*, Nucl. Acids Res. 18:2699-706 (1990); Sheffield *et al.*, Proc. Natl. Acad. Sci. USA 86:232-6 (1989)).

**[0075]** A polymerase-mediated primer extension method may also be used to identify the polymorphism(s). Several such methods have been described in the patent and scientific literature and include the "Genetic Bit Analysis" method (WO 92/15712) and the ligase/polymerase mediated genetic bit analysis (U.S. Pat. No.

5,679,524. Related methods are disclosed in WO 91/02087, WO 90/09455, WO 95/17676, and U.S. Pat. Nos. 5,302,509 and 5,945,283. Extended primers containing the complement of the polymorphism may be detected by mass spectrometry as described in U.S. Pat. No. 5,605,798. Another primer extension method is allele-specific PCR (Ruano *et al.*, 1989, supra; Ruano *et al.*, 1991, supra; WO 93/22456; Turki *et al.*, J. Clin. Invest. 95:1635-41 (1995)).

**[0076]** Another technique, which may be used to analyze gene expression and polymorphisms, includes multicomponent integrated systems, which miniaturize and compartmentalize processes such as PCR and capillary electrophoresis reactions in a single functional device. An example of such technique is disclosed in U.S. Pat. No. 5,589,136, the disclosure of which is incorporated herein by reference in its entirety, which describes the integration of PCR amplification and capillary electrophoresis in chips.

**[0077]** Quantitative PCR and digital PCR can be used to measure the level of a polynucleotide in a sample. Digital Polymerase Chain Reaction (digital PCR, dPCR or dePCR) can be used to directly quantify and clonally amplify nucleic acids including DNA, cDNA or RNA. Digital PCR amplifies nucleic acids by temperature cycling of a nucleic acid molecule with a DNA polymerase. The reaction is typically carried out in the dispersed phase of an emulsion capturing each individual nucleic acid molecule present in a sample within many separate chambers or regions prior to PCR amplification. A count of chambers containing detectable levels of PCR end-product is a direct measure of the absolute nucleic acids quantity.

**[0078]** Quantitative polymerase chain reaction (qPCR) is a modification of the polymerase chain reaction and real-time quantitative PCR are useful for measuring the amount of DNA after each cycle of PCR by use of fluorescent markers or other detectable labels. Quantitative PCR methods use the addition of a competitor RNA (for reverse-transcriptase PCR) or DNA in serial dilutions or co-amplification of an internal control to ensure that the amplification is stopped while in the exponential growth phase.

**[0079]** Modifications of PCR and PCR techniques are routine in the art and there are commercially available kits useful for PCR amplification.

**[0080]** The detectable label may be a radioactive label or may be a luminescent, fluorescent of enzyme label. Indirect detection processes typically comprise probes covalently labeled with a hapten or ligand such as digoxigenin (DIG) or biotin. In one aspect, following the hybridization step, the target-probe duplex is detected by an antibody- or streptavidin-enzyme complex. Enzymes commonly used in DNA diagnostics are horseradish peroxidase and alkaline phosphatase. Direct detection methods include the use of fluorophor-labeled oligonucleotides, lanthanide chelate-labeled oligonucleotides or oligonucleotide-enzyme conjugates. Examples of fluorophor labels are fluorescein, rhodamine and phthalocyanine dyes.

**[0081]** Examples of detection modes contemplated for the disclosed methods include, but are not limited to, spectroscopic techniques, such as fluorescence and UV-Vis spectroscopy, scintillation counting, and mass spectroscopy. Complementary to these modes of detection, examples of labels for the purpose of detection and quantitation used in these methods include, but are not limited to, chromophoric labels, scintillation labels, and mass labels. The expression levels of polynucleotides and polypeptides measured using these methods may be normalized to a control established for the purpose of the targeted determination.

**[0082]** Label detection will be based upon the type of label used in the particular assay. Such detection methods are known in the art. For example, radioisotope detection can be performed by autoradiography, scintillation counting or phosphor imaging. For hapten or biotin labels, detection is with an antibody or streptavidin bound to a reporter enzyme such as horseradish peroxidase or alkaline phosphatase, which is then detected by enzymatic means. For fluorophor or lanthanide-chelate labels, fluorescent signals may be measured with spectrofluorimeters with or without time-resolved mode or using automated microtitre plate readers. With enzyme labels, detection is by color or dye deposition (p-nitropheny phosphate or 5-bromo-4-chloro-3-indolyl

phosphate/nitroblue tetrazolium for alkaline phosphatase and 3,3'-diaminobenzidine-$NiCl_2$ for horseradish peroxidase), fluorescence (*e.g.*, 4-methyl umbelliferyl phosphate for alkaline phosphatase) or chemiluminescence (the alkaline phosphatase dioxetane substrates LumiPhos 530 from Lumigen Inc., Detroit Mich. or AMPPD and CSPD from Tropix, Inc.). Chemiluminescent detection may be carried out with X-ray or polaroid film or by using single photon counting luminometers.

**[0083]** In another aspect of this disclosure, expression levels of proteins comprising SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42 and/or 44 can be measured and quantitated using techniques known in the art including, for example, Western blots, ELISA assays and the like. The term "polypeptide" or "polypeptides" is used interchangeably with the term "protein" or "proteins" herein.

**[0084]** In another embodiment, a method for protein expression profiling comprises using one or more (*e.g.*, a plurality of) antibodies to one or more biomarkers for measuring targeted polypeptide levels from a biological sample. In one embodiment contemplated for the method, the antibodies for the panel are bound to a solid support. The method for protein expression profiling may use a second antibody having specificity to some portion of the bound polypeptide. Such a second antibody may be detectably labeled with molecules useful for detection and quantitation of the bound polypeptides. Additionally, other reagents are contemplated for detection and quantitation including, for example, small molecules such as cofactors, substrates, complexing agents, and the like, or large molecules, such as lectins, peptides, olionucleotides, and the like. Such moieties may be either naturally occurring or synthetic.

**[0085]** The disclosure further contemplates, antibodies capable of specifically binding to a biomarker polypeptides encoded in proper frame, based upon transcriptional and translational starts, of the above-identified polynucleotide biomarker sequences (*e.g.*, comprising SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, or 43). The disclosure thus includes isolated, purified, and recombinant polypeptides

comprising a contiguous span of at least 4 amino acids, typically at least 6, more commonly at least 8 to 10 amino acids encoded by a polynucleotide comprising SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43.

**[0086]** The disclosure also contemplates the use of immunoassay techniques for measurement of polypeptide biomarkers identified herein. The polypeptide biomarker can be isolated and used to prepare antisera and monoclonal antibodies that specifically detect a biomarker gene product. Mutated gene products also can be used to immunize animals for the production of polyclonal antibodies. Recombinantly produced peptides can also be used to generate antibodies. For example, a recombinantly produced fragment of a polypeptide can be injected into a mouse along with an adjuvant so as to generate an immune response. Murine immunoglobulins which bind the recombinant fragment with a binding affinity of at least $1x10^{7}$ $M^{-1}$ can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells. The bank of hybridomas can be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an affinity of at least $1x10^{6}$ $M^{-1}$. More specifically, immunoglobulins that selectively bind to the variant polypeptides but poorly or not at all to wild-type polypeptides are selected, either by pre-absorption with wild-type proteins or by screening of hybridoma cell lines for specific idiotypes that bind the variant, but not wild-type, polypeptides.

**[0087]** Polynucleotides capable of expressing the polypeptides can be generated using techniques skilled in the art based upon the identified sequences herein. Such polynucleotides can be expressed in hosts, wherein the polynucleotide is operably linked to (*i.e.*, positioned to ensure the functioning of) an expression control sequence. Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosome. Expression vectors can contain selection markers (*e.g.*, markers based on tetracyclin resistance or hygromycin resistance)

to permit detection and/or selection of those cells transformed with the desired polynucleotide.

**[0088]** Polynucleotides encoding a variant polypeptide may include sequences that facilitate transcription and translation of the coding sequences such that the encoded polypeptide product is produced. Construction of such polynucleotides is known in the art. For example, such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts, and, optionally, sequences necessary for replication of a vector.

**[0089]** Prokaryotes can be used as host cells for the expression of a variant polypeptides, such techniques are known in the art. Other microbes, such as yeast, may also be used for expression. In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce polypeptides of the disclosure. Eukaryotic cells useful in the methods of the disclosure include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, Jurkat cells, and so forth. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, an necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

**[0090]** The techniques for polynucleotide cloning and expression are useful in the disclosure for the generation of probes capable of hybridizing to polynucleotide biomarkers or the generation of antibodies useful for binding polypeptide biomarkers of the disclosure.

**[0091]** In further methods, peptides, drugs, fatty acids, lipoproteins, or small molecules which interact with a biomarker (*e.g.*, a polynucleotide or polypeptide, protein, or a fragment comprising a contiguous span of at least 4 amino acids, at least 6 amino acids, or typically at least 8 to 10 amino acids or more of sequences corresponding to the biomarkers herein) can be used as detection agents for measuring biomarkers. The molecule to be tested for binding is labeled with a detectable label, such as a

fluorescent, radioactive, or enzymatic tag. After removal of non-specifically bound molecules, bound molecules are detected using appropriate means.

**[0092]** These results, with reference to the figures and specific examples below, demonstrate that it is possible to sample cells through a minimally invasive swabbing collection method from an area distant from a cancerous lesion, but capable of indicating a non-normal colon condition. In that regard, samples taken either minimally invasively or non-invasively would render samples that could be analyzed using the disclosed panel of biomarkers. Such non-invasive procedures not only reduce the cost of determination of CRC, but reduce the discomfort and risk associated with current methodology. All these factors together increase the attractiveness of regular testing, and hence patient compliance. Increased patient compliance, coupled with an effective determination for CRC, enhance the prospects for early detection, and enhanced survival rates.

**[0093]** Table 3 below demonstrates the differences in expression profiles based upon biomarkers of the disclosure. FHSH refers to family and self history of the subject. FHSH subjects lacked a history of polyps. As referenced in table 3, "Others" refer to subjects that have a history of gastrointestinal diseases or disorders. Accordingly, in one aspect of the disclosure, a predictive biomarker for gastrointestinal inflammatory disease or disorder would include detecting a change in expression of IL-8, CD44, c-myc, and/or P21, which all show larger changes (*e.g.*, about 19, 63, 50 and 56%, respectively, relative to controls). It is important to note that a change in expression of a biomarker of the disclosure need not necessarily be an increase in expression relative to a control. Rather, a change can be an increase or decrease relative to a control so long as the change represents a statistically significant difference relative to the control. In one aspect, the change is at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more in an increase or decrease relative to a control. Where a panel of biomarkers are used in the detection of a disease or disorder, a smaller change relative to a control can be indicative of the disease or disorder

or risk thereof in comparison to a change in each biomarker alone. A statistician of skill in the art will be capable of identifying statistically significant differences in a biomarker or panel of biomarkers relative to a control value(s).

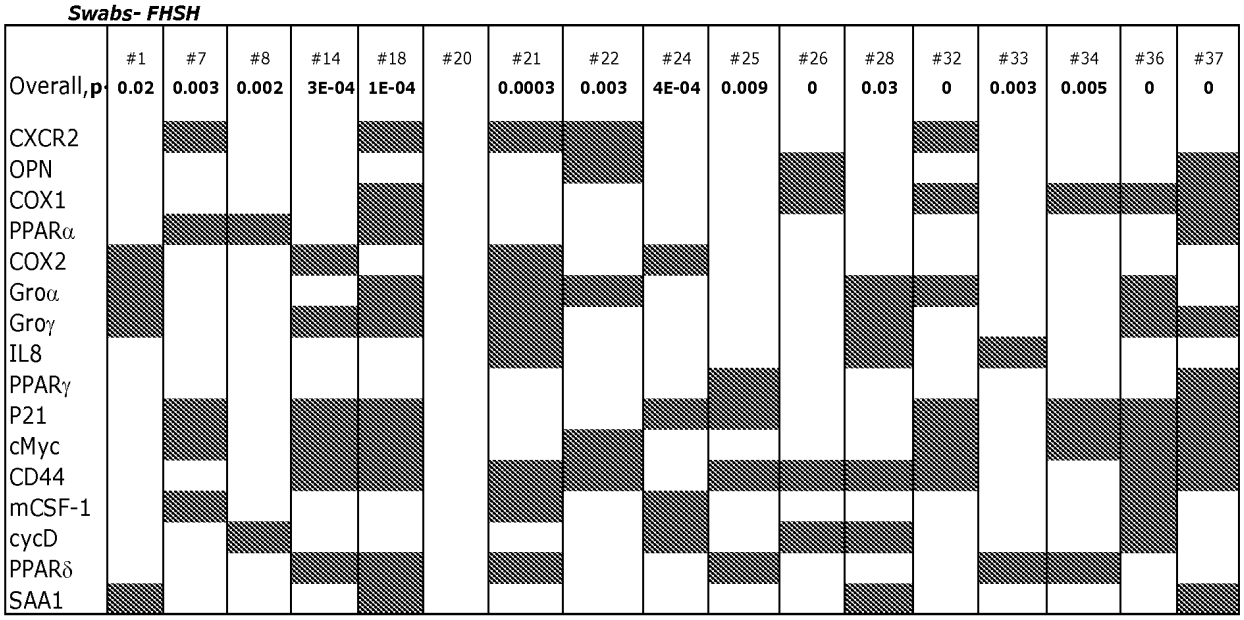
**[0094]** In another aspect, the disclosure provides methods of early detection or diagnosis of a colorectal cancer or gastrointestinal inflammatory disease or disorder based upon measurement of any of the biomarkers in table 3 by rectal or buccal swabs. This method can be followed by a determination at a later time by measuring the same, or one or more additional, biomarkers. For example, early detection or diagnosis can be based upon screening changes in any one or more of the biomarkers described, wherein an increase in a biomarker's expression (*e.g.*, IL-8, P21, c-myc, and/or CD44) is indicative of a gastrointestinal inflammatory disease or disorder or the risk of acquiring an gastrointestinal inflammatory disease or disorder; following initial diagnosis or prediction the same or different makers (*e.g.*, IL-8) can be measured to determine the prognosis or development of a disease. The data below indicate, for example, that the biomarker IL-8 and OPN may be indicative of later stage development of a gastrointestinal disease or disorder.

Table 3

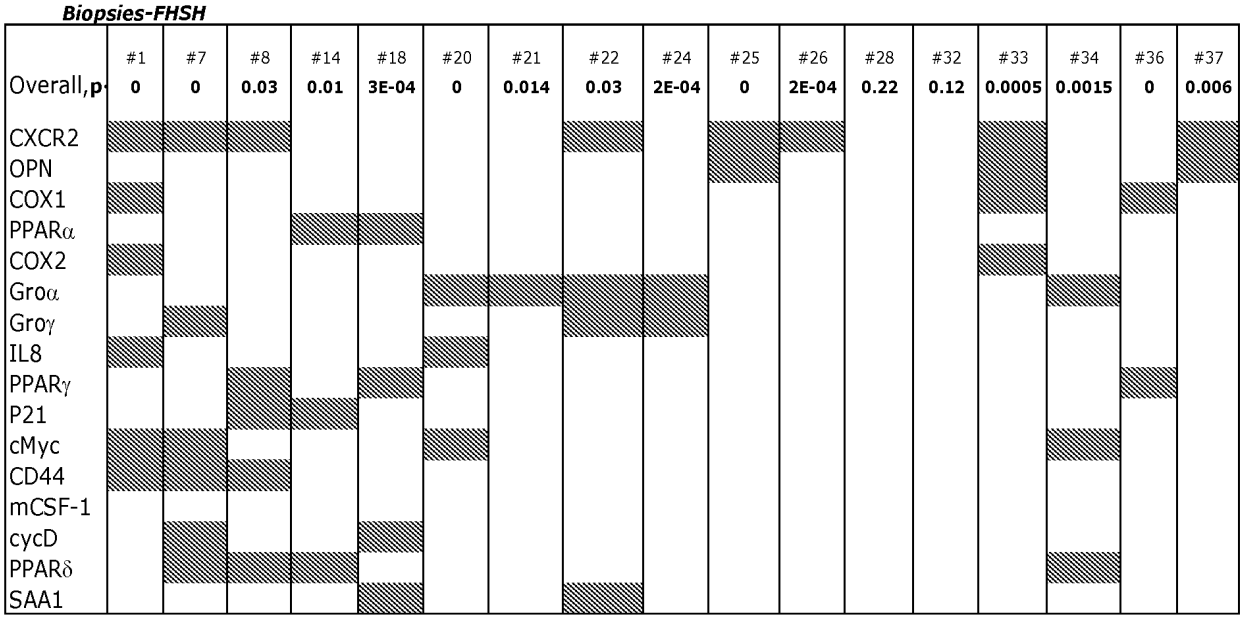
| | ***Swabs*** **FHSH,n=16** **p<0.0000** | ***Swabs*** **Others, n=9** **p<0.0000** | ***Biopsies*** **FHSH, n=17** **p<0.0001** | ***Biopsies*** **Others, n=8** **p<0.0001** |
|---|---|---|---|---|
| Overall | | | | |
| CXCR2 | **31%** | **56%** | **57%** | **38%** |
| OPN | **19** | **44** | **18** | **63** |
| COX1 | **38** | **33** | **18** | **13** |
| PPARα | **25** | **22** | **12** | **13** |
| COX2 | **25** | **44** | **12** | **13** |
| Groα | **44** | **56** | **29** | **25** |
| Groγ | **44** | **56** | **17** | **25** |
| IL8 | **19** | **67** | **12** | **13** |
| PPARγ | **13** | **33** | **17** | **25** |
| P21 | **56** | **78** | **12** | **25** |
| cMyc | **50** | **56** | **29** | **13** |
| CD44 | **63** | **67** | **17** | **13** |
| mCSF-1 | **25** | **33** | **0** | **0** |
| cycD | **31** | **44** | **12** | **0** |
| PPARδ | **38** | **56** | **24** | **50** |
| SAA1 | **25** | **22** | **12** | **25** |

**[0095]** Table 4 gives specifics regarding profiling of subjects based upon the methods of the disclosure.

***Swabs- FHSH***




***Biopsies-FHSH***




**[0096]** Methods and kits for the polynucleotide and polypeptide expression profiling for the panel of molecular markers are also contemplated as part of the present disclosure.

**[0097]** In one embodiment, a kit for gene expression profiling comprises the reagents and instructions necessary for the gene expression profiling of the biomarkers or biomarker panel. Thus,

for example, the reagents may include primers, enzymes, and other reagents for the preparation, detection, and quantitation of cDNAs for the claimed panel of biomarkers. The primers listed in SEQ ID NOs: 45-88 are particularly suited for use in gene expression profiling using RT-PCR based on the claimed panel. The primers listed in SEQ ID NOs: 45-88 were specifically designed, selected, and tested accordingly. In addition to the primers, reagents such as dinucleotide triphosphate comprising dinucleotide triphosphates (*e.g.*, dATP, dGTP, dCTP, and dTTP), reverse transcriptase, and a thermostable DNA polymerase. Additionally buffers, inhibitors and activators used for the RT-PCR process are suitable reagents for inclusion in the kit embodiment. Once the cDNA has been sufficiently amplified to a specified end point, the cDNA sample must be prepared for detection and quantitation. One method contemplated for detection of polynucleotides is fluorescence spectroscopy using fluorescent moieties or labels that are suited to fluorescence spectroscopy are desirable for labeling polynucleotides and may also be included in reagents of the kit embodiment.

**[0098]** In one embodiment, the disclosure provides a kit useful for identifying biomarkers indicative of a gastrointestinal disease or disorder. For example, the kit of the disclosure can comprise a of one or more oligonucleotides designed for identifying alleles and/or biomarkers of the disclosure. In another embodiment, the kit further comprises a manual with instructions for (a) performing one or more reactions on a human nucleic acid sample to identify biomarkers and/or alleles present in the subject.

**[0099]** The oligonucleotides in a kit of the disclosure may also be immobilized on or synthesized on a solid surface such as a microchip, bead, or glass slide (see, *e.g.*, WO 98/20020 and WO 98/20019). Such immobilized oligonucleotides may be used in a variety of detection assays, including but not limited to, probe hybridization and polymerase extension assays. Immobilized oligonucleotides useful in practicing the disclosure may comprise an ordered array of oligonucleotides designed to rapidly screen a nucleic acid sample.

**[00100]** Kits of the disclosure may also contain other components such as hybridization buffer (*e.g.*, where the oligonucleotide probes) or dideoxynucleotide triphosphates (ddNTPs; *e.g.*, for primer extension). In one embodiment, the set of oligonucleotides consists of primer-extension oligonucleotides. The kit may also contain a polymerase and a reaction buffer optimized for primer-extension mediated by the polymerase. Kits may also include detection reagents, such as biotin- or fluorescent-tagged oligonucleotides or ddNTPs and/or an enzyme-labeled antibody and one or more substrates that generate a detectable signal when acted on by the enzyme. It is also contemplated that the above described methods and compositions of the disclosure may be utilized in combination with other biomarker techniques.

**[00101]** Nucleic acid samples, for example for use in variance identification, can be obtained from a variety of sources as known to those skilled in the art, or can be obtained from genomic or cDNA sources by known methods.

**[00102]** In another embodiment, a kit for protein expression profiling comprises the reagents and instructions necessary for protein expression profiling of a polypeptide biomarker panel. Thus, in this embodiment, the kit for protein expression profiling includes supplying an antibody panel based on a panel of biomarkers for measuring targeted polypeptide levels from a biological sample. One embodiment contemplated for such a panel includes the antibody panel bound to a solid support. Additionally, the reagents included with the kit for protein expression profiling may use a second antibody having specificity to some portion of the bound polypeptide. Such a second antibody may be labeled with molecules useful for detection and quantitation of the bound polypeptides.

**[00103]** Methods for diagnostic tests are well known in the art. Generally, the diagnostic test of the disclosure involves determining whether an individual has a variance or variant form of a gene or a change in expression.

**[00104]** Integrated systems can be envisaged mainly when microfluidic systems are used. These systems comprise a pattern of microchannels designed onto a glass, silicon, quartz, or plastic wafer included on a microchip. The movements of the samples are

controlled by electric, electroosmotic or hydrostatic forces applied across different areas of the microchip. The microfluidic system may integrate nucleic acid amplification, microsequencing, capillary electrophoresis and a detection method such as laser-induced fluorescence detection.

**[00105]** It is also contemplated that the gene expression profile may be transmitted to a remote location for analysis. For example, changes in a detectable signal related to gene expression from a first time and a second time are communicated to a remote location for analysis.

**[00106]** The digital representation of the detectable signal is transmittable over any number of media. For example, such digital data can be transmitted over the Internet in encrypted or in publicly available form. The data can be transmitted over phone lines, fiber optic cables or various air-wave frequencies. The data are then analyzed by a central processing unit at a remote site, and/or archived for compilation of a data set that could be mined to determine, for example, changes with respect to historical mean "normal" values of a genetic expression profile of a subject.

**[00107]** Embodiments of the disclosure include systems (*e.g.*, internet based systems), particularly computer systems which store and manipulate the data corresponding to the detectable signal obtained an expression profile. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to analyze the digital representative of an expression profile or plurality of profiles. The computer system typically includes a processor for processing, accessing and manipulating the data. The processor can be any well-known type of central processing unit.

**[00108]** Typically the computer system is a general purpose system that comprises the processor and one or more internal data storage components for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

**[00109]** In one particular embodiment, the computer system includes a processor connected to a bus which is connected to a

main memory (preferably implemented as RAM) and one or more internal data storage devices, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system further includes one or more data retrieving device for reading the data stored on the internal data storage devices.

**[00110]** The data retrieving device may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, or a modem capable of connection to a remote data storage system (*e.g.*, via the internet) and the like. In some embodiments, the internal data storage device is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, and the like, containing control logic and/or data recorded thereon. The computer system may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

**EXAMPLES**

**[00111]** The genes in the expression panel fall into four major groups: 1) APC/b-catenin pathway, including c-myc, cyclin D1, and proliferating peroxisome activating receptor (PPAR alpha, delta and gamma); 2) NF-kB/inflammation pathway, including the growth-related oncogenes (Gro)-alpha and gamma osteopontin (OPN), and colony-stimulating factor (M-CSF-1), cyclo-oxygenases (COX)-1 and 2, interleukin-8 (IL-8), and the cytokine receptor CXCR2; 3) cell cycle/transcription factors, including p21, cyclin D1, c-myc, PPAR alpha, delta and gamma and 4) cell communication signals, including IL-8, PPAR alpha, delta and gamma, CXCR2, CD44, and OPN.

**[00112]** Biopsies of colonic mucosa, from rectosigmoid or rectal areas, were taken from subjects during the course of colonoscopy. The subjects included individuals with adenomatous polyps, the precursor of most colon cancers; individuals with a family history or self history of cancer; and individuals with no polyps or family/self history, who served as normal controls. In all cases, the biopsies were composed of normal appearing mucosa.

**[00113]** Rectal mucosal samples were obtained from individuals in all these groups by a rectal smear, using a small anoscope. A

small brush was inserted through the anoscope several centimeters into rectum, and cells removed by gentle scraping. In addition, buccal mucosal samples were obtained by swabbing the buccal cavity about 3-5 centimeters into the buccal cavity (typically at the back of the cheek).

**[00114]** Total RNA was extracted from each tissue sample or swab, and reverse transcriptase used to convert RNA to cDNA. The expression of each of a plurality of genes was then determined using PCR, with primers designed to amplify each gene.

**[00115]** The data presented in Figure 1 and 2 demonstrate the ability to determine a risk or presence of a colorectal cancer by obtaining swabs from the buccal cavity of a subject. Such swab techniques are beneficial and promote patient compliance because they cause less discomfort than colonscopy, rectal swabs and bioposies. Information comparing rectal swabs vs. biopsies as a means of tissue collection, in about 90 individuals, 37 individuals with history, 25 individuals with polyps (with or without history), and 23 controls with no polyps, no family or self history of cancer, and no known obvious upper GI problems. In this 90 patient study there was no cancer in situ case, 5 individuals scheduled for surgery due to colon cancer were swabbed.

**[00116]** The statistical approach uses a global multivariate analysis of variance (ANOVA), that takes into account correlations among the expression levels of different genes. This type of analysis controls the false positive rate by providing a single test of whether the expression patterns, based on all the genes in the subset, differ between groups or individuals. If the global test is significant for a particular individual or for a particular group, a univariate test was then used to determine which genes are contributing to the global difference.

**[00117]** This was supplemented by an analysis based on Mahalanobis-distance (M-dist). M-dist is a multivariate measure of the distance between a single gene expression value from a patient and the mean of a pool of samples from controls. M-dist is expected to have a chi-square distribution with degrees of freedom equal to the number of genes. An arbitrary cut-off point, such as the 95th percentile, is chosen, below which most individual control values

will fall. Thus an experimental subject with an M-dist sample value above this criterion can be thought of as being significantly different from a control sample.

**[00118]** M-dist values can be determined for either each individual biopsy or swab removed from an individual, or for the mean of gene expression values from all samples taken from an individual. These M-dist values can then be plotted on a graph, with the value from each sample or each individual represented by a single point. The sensitivity and specificity of the approach can be readily visualized from these plots. The sensitivity is the proportion of values in the experimental group that are above the 95th percentile--represented as a horizontal line on the graph--while the specificity is the proportion of all values above the line which belong to individuals in the experimental group.

**[00119]** Mahalanobis (M-dist) was selected as the measure of statistical significance because it summarizes in a single number the differences between a pattern of gene expression for any individual against the average of a pool of individuals, taking into account variability of each gene's expression and correlations among pairs of genes. This allowed us to determine on a probability scale, how different one gene expression pattern is from another. First, for each control biopsy, The M-dist was calculated from the multivariate mean of the other normal control biopsies. Then an M-dist was computed for each biopsy from each individual with polyps, family/self history of cancer, in which M-dist measured the individual's multivariate distance (*i.e.*, difference in pattern of expression) from the pooled mean of the normal control samples. Using this approach, one can determine an upper bound for the normal controls, at any arbitrary level of significance, such as the 95th percentile. This allows analysis of significance of gene expression values of any individual experimental patient compared with the pool of normal controls.

**[00120]** Figure 1 shows the Mahalanobis distance for biopsy samples, taken from (left to right), controls, resected colon cancer, individuals with family history, and individuals with polyps. Each circle represents the M-distance of a single tissue sample, and all the circles in a single vertical line represent

samples from a single individual. The horizontal line represents an M-dist corresponding to the 95th percentile for normal controls, so that any values above this line are significantly different from the pooled normal control values at a significance level of $p < 0.05$ (*i.e.* result is not like that for normal controls).

**[00121]** As expected, most of the samples from control individuals (99/104) fell below the 95th percentile. Four out of seventeen individuals had at least one sample above the line, and just one 1/17 had two samples. In contrast, all biopsy samples from resected colon cancer tissue had M-dist values above the 95th percentile, and for 6/7 individuals, each value was far above the line ($p < 0.001$). For individuals with family history and individuals with polyps, some samples were above the 95th percentile and some below it, but all 13 individuals with family history had at least one sample above the line, as did 21/24 (87.5%) individuals with polyps. Ten of thirteen (77%) individuals with family history had more than one biopsy with an M-dist value above the line, while 14/24 (58%) individuals with polyps did.

**[00122]** Figure 1B shows an analysis carried out on a second patient pool, one including individuals with no polyps or family/self history (Control), individuals with family history, individuals with polyps. The results are similar to those of the earlier study. All of the control biopsies had M-dist values below the 95th percentile. Fifteen of eighteen (83%) individuals with family history had at least one value above this percentile, while 4/9 (44%) individuals with polyps did.

**[00123]** Figure 1C shows the same analysis carried out on rectal smear samples taken from the same individuals used in the study presented in Figure 1B. All but one normal control biopsy were at or below the 95th percentile. 15/17 (88%) individuals with family/self history had at least one M-dist value above the 95th percentile, and 13/17 (76.5%) had at least two values above it. All 9 individuals with polyps had at least one value above the 95th percentile, and 5/9 (56%) had at least two values above this criterion. In addition, all smear taken from known colon cancer from two individuals had M-dist values far above the 95th percentile.

**[00124]** Figure 2A-B show the similar analysis based upon a swab. Figure 2A shows a 90 patient study of gene expression values for 16 genes from each subject, controls tend to fall below the 95% chi-square distribution line. A tendency of subjects with cancer fall above the line can be seen at the far right. Figure 2B shows the 95% chi-square distribution of gene analysis from buccal swabs of 21 controls and 8 cancer subjects. The data demonstrate that a buccal swab and analysis of a panel of genes in the sample can be used to identify subject with a gene expression profile different than that a normal control. The difference being indicative of a risk factor for colorectal cancer.

**[00125]** Colon cancer is the result of a progression of molecular and cellular changes in the mucosal tissue lining the colon. While these changes are not completely understood, they are accompanied by alterations in the expression levels of many genes. Taking advantage of this fact, we have previously shown that normal appearing colon mucosa from individuals with polyps, family/self history of cancer has a different expression profile. The tissue samples from these studies were obtained by colonoscopy, but here we have shown that samples can also be obtained by rectal smear, a non-invasive procedure that can be carried out quickly and cheaply in any physician's office, without bowel preparation or anesthesia.

**[00126]** These results indicate that one can identify all cases of colon cancer and distinguish a high % of individuals with adenomatous polyps from those without polyps. Individuals at risk for cancer can be recommended for colonoscopies, while those with no risk may choose to avoid this costly and invasive procedure.

**[00127]** A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the description. Accordingly, other embodiments are within the scope of the following claims.

**What is claimed is**

1. A method of diagnosing a colorectal cancer or gastrointestinal inflammatory disease or disorder in an asymptomatic subject, comprising:

(i) collecting mucosal epithelial cells from the buccal area of the subject;

(ii) measuring the expression level of at least two polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, and 43, in the collected cells;

(iii) comparing the expression level of the at least two polynucleotides to the level of the same polynucleotides in a normal control,

wherein a change in the expression level compared to the normal control is indicative of colorectal cancer or an inflammatory disease or disorder.

2. A method of diagnosing a colorectal cancer or an inflammatory disease or disorder of an asymptomatic subject, comprising:

(i) swabbing the buccal area of the subject to collect epithelial cells;

(ii) measuring the expression level of at least two polynucleotides comprising a sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, and 43 in the collected cells;

(iii) comparing the expression level of the at least two polynucleotide to the level of the same polynucleotides in a normal control,

wherein a change in the expression level compared to the normal control is indicative of colorectal cancer or an inflammatory disease or disorder.

3. The method of claim 1 or 2, wherein the at least two polynucleotides comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more polynucleotides.

4. The method of claim 3, wherein the at least two polynucleotides each encode a polypeptide selected from the group consisting of CXCR2, OPN, COX1, PPARα, COX2, IL8, P21, c-Myc,CD44,

and PPARδ.

5. The method of claim 3, wherein the change is an increase in the expression level.

6. The method of claim 1 or 2, wherein a plurality of probes is used to detect the expression level of the at least two polynucleotides.

7. The method of claim 6, wherein the plurality of probes hybridize to a set of polynucleotides selected from the group consisting of:

(a) a first polynucleotide comprising SEQ ID NO:3 and a second polynucleotide comprising SEQ ID NO:5;

(b) a first polynucleotide comprising SEQ ID NO:3, a second polynucleotide comprising SEQ ID NO:5 and a third polynucleotide comprising SEQ ID NO:17; or

(c) a first polynucleotide comprising SEQ ID NO:3, a second polynucleotide comprising SEQ ID NO:5, a third polynucleotide comprising SEQ ID NO:9 or 11, and a fourth polynucleotide comprising SEQ ID NO:17.

8. The method of claim 6, wherein the plurality of probes comprise a sequence selected from the group consisting of SEQ ID NO:45-87 or 88.

9. The method of claim 6, wherein the plurality of probes are detectably labeled.

10. The method of claim 8, wherein the plurality of probes are used to amplify the at least two polynucleotides.

11. The method of claim 1 or 2, wherein the inflammatory disease or disorder is selected from the group consisting of pseudomembranous colitis, hemorrhagic colitis, hemolytic-uremic syndrome colitis, collagenous colitis, ischemic colitis, radiation colitis, drug and chemically induced colitis, diversion colitis, ulcerative colitis, irritable bowel syndrome, irritable colon syndrome, Barrett's disease and Crohn's disease.

12. The method of claim 11, wherein the Crohn's disease is selected from the group consisting of active, refractory, and fistulizing Crohn's disease.

13. The method of claim 1, wherein the collecting of epithelial cells is done by swabbing the buccal or rectal area of

the subject.

14. A method of diagnosing a colorectal cancer or an inflammatory disease or disorder in a subject, comprising:

(a) contacting a buccal or rectal sample from a subject with at least two probes comprising at least 8 contiguous nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43; and

(b) quantifying the amount of polynucleotide molecules that hybridizes to the at least two probes,

wherein an increase in the amount of polynucleotides relative to a normal control is indicative of the subject having a colorectal cancer or a gastrointestinal disease or disorder, and

wherein the subject has no familial or self history of a gastrointestinal disease or disorder.

15. A method of screening a subject for the risk of developing a colorectal cancer or a gastrointestinal disease or disorder, comprising:

(a) contacting a buccal or rectal sample from the subject with at least two probes comprising at least 8 contiguous nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43; and

(b) quantifying the amount of polynucleotide molecules that hybridizes to the at least two probes,

wherein an increase in the amount of polynucleotides relative to a control is indicative of the subject having a risk of developing a colorectal cancer or gastrointestinal disease or disorder, and

wherein the subject has no familial or self history of a colorectal cancer or a gastrointestinal disease or disorder.

16. The method of claim 14 or 15, wherein the at least two probes comprises three or more probes.

17. The method of claim 14 or 15, wherein the at least two probes comprise a panel that hybridizes to the following set of polynucleotides:

(a) SEQ ID NO:3 and SEQ ID NO:5;

(b) SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:17; or

(c) SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 or 11, and SEQ ID

NO:17.

18. The method of claim 14 or 15, further comprising monitoring the prognosis of the subject comprising monitoring the expression profile of at least two polynucleotides biomarker selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, and SEQ ID NO:17,

wherein the expression profile is monitored at a plurality of time points.

19. The method of claim 14 or 15, wherein the at least two probes comprise two sequences selected from the group consisting of SEQ ID NO:45-87 or 88.

20. The method of claim 14 or 15, wherein the gastrointestinal disease or disorder is an inflammatory disease or disorder selected from the group consisting of pseudomembranous colitis, hemorrhagic colitis, hemolytic-uremic syndrome colitis, collagenous colitis, ischemic colitis, radiation colitis, drug and chemically induced colitis, diversion colitis, ulcerative colitis, irritable bowel syndrome, irritable colon syndrome, Barrett's disease and Crohn's disease.

21. The method of claim 20, wherein the Crohn's disease is selected from the group consisting of active, refractory, and fistulizing Crohn's disease.

22. A method comprising:

(a) providing a sample comprising polypeptides obtained from a buccal sample of a subject;

(b) contacting the sample with at least two probes that specifically bind to at least two polypeptides consisting of a sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, or 44; and

(c) determining the level of the at least two polypeptides in the sample,

wherein an increase in the amount of polypeptides relative to a normal control is indicative of the subject having or at risk of having a colorectal cancer or gastrointestinal disease or disorder.

23. The method of claim 22, wherein the sample is obtained

by swabbing the buccal area of the subject.

24. The method of claim 22, wherein the subject has no familial or self history of a colorectal cancer or gastrointestinal disease or disorder.

25. The method of claim 22, wherein the at least two polypeptides are selected from the group consisting of CXCR2, OPN, COX1, PPARα, COX2, IL8, P21, c-Myc, CD44, and PPARδ.

26. The method of claim 22, wherein the inflammatory disease or disorder is selected from the group consisting of pseudomembranous colitis, hemorrhagic colitis, hemolytic-uremic syndrome colitis, collagenous colitis, ischemic colitis, radiation colitis, drug and chemically induced colitis, diversion colitis, ulcerative colitis, irritable bowel syndrome, irritable colon syndrome, Barrett's disease and Crohn's disease.

27. The method of claim 26, wherein the Crohn's disease is selected from the group consisting of active, refractory, and fistulizing Crohn's disease.

28. The method of claim 22, wherein the at least two probes comprises 3, 4, 5, 6, 7, 8, 9, 10 or more probes.

29. The method of claim 22, wherein the probe comprises an antibody.

30. A kit for carrying-out the method of claim 1, 2, 14, 15, or 22.

31. The kit of claim 30, comprising an oligonucleotide probe or primer pair for detecting a polynucleotide comprising a sequence as set forth in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37, 39, 41, or 43.

32. The kit of claim 30, comprising an agent the specifically detects a polypeptide comprising a sequence as set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42 or 44.

33. The kit of claim 30, comprising a buccal swab.

34. The kit of claim 30, comprising a buccal swab device.

35. A method comprising:

measuring the expression profile of at least two genes selected from the group consisting of selected from the group

consisting of CXCR2, OPN, COX1, PPARα, COX2, IL8, P21, c-Myc, CD44, and PPARδ present in the buccal swab from a subject; and

transmitting the expression profile to a technician or a caregiver.

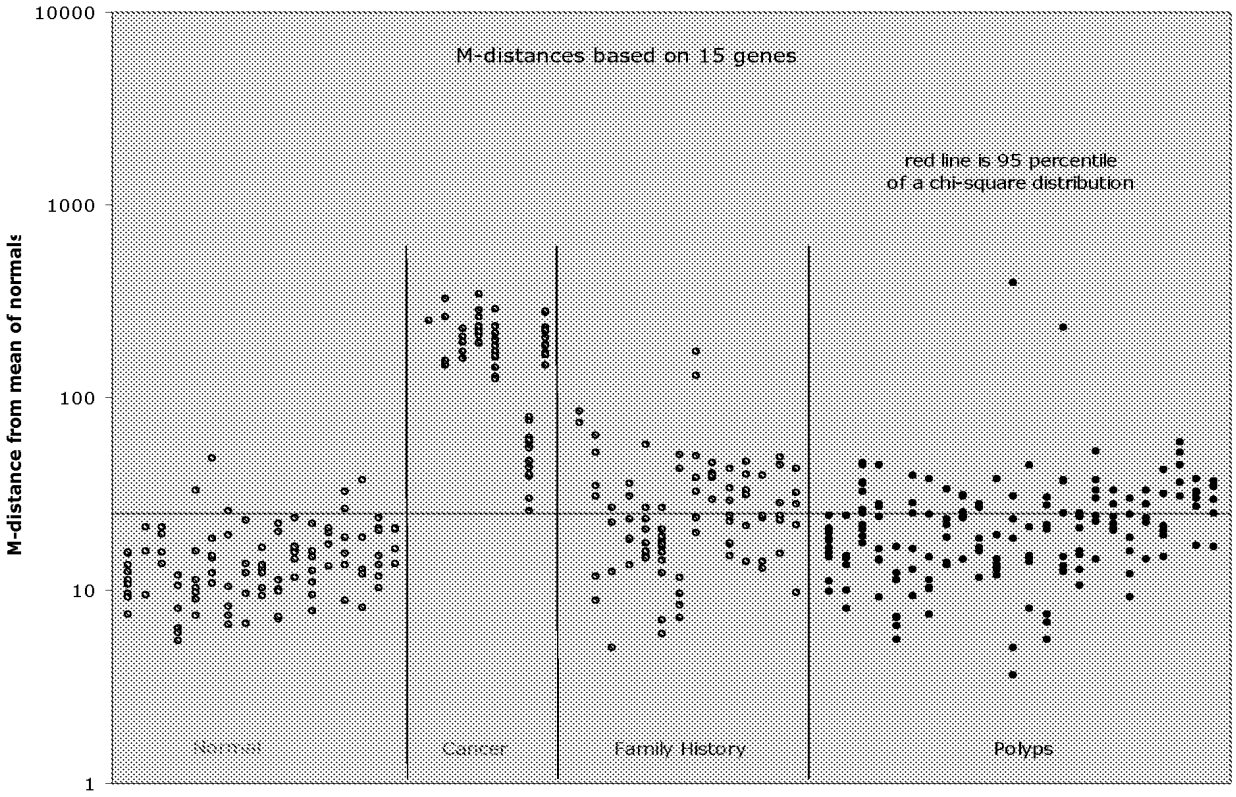



FIGURE 1A

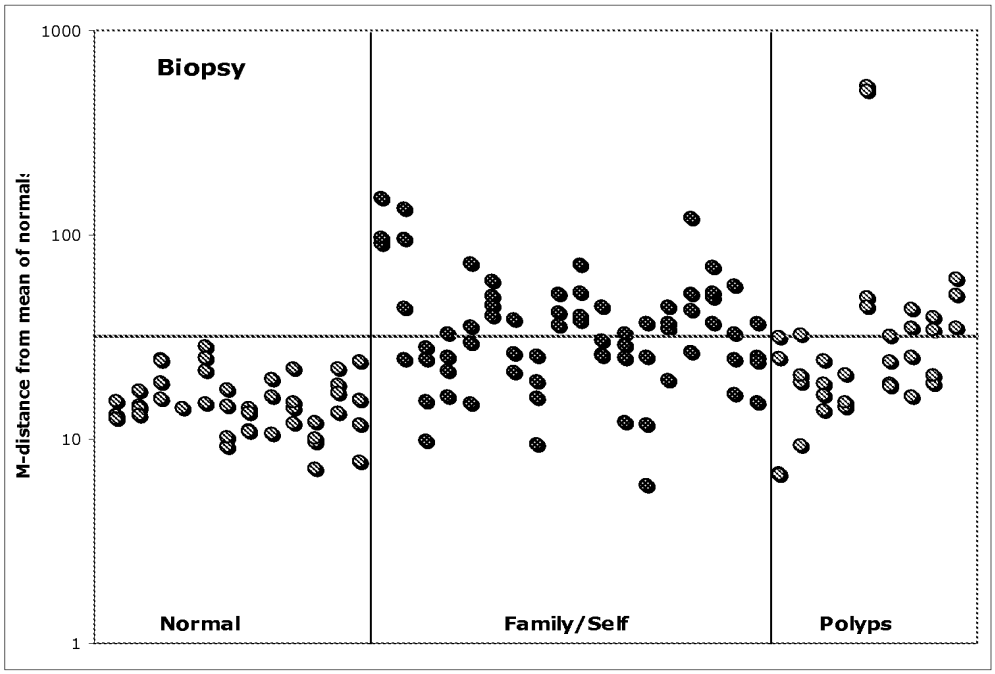



FIGURE 1B

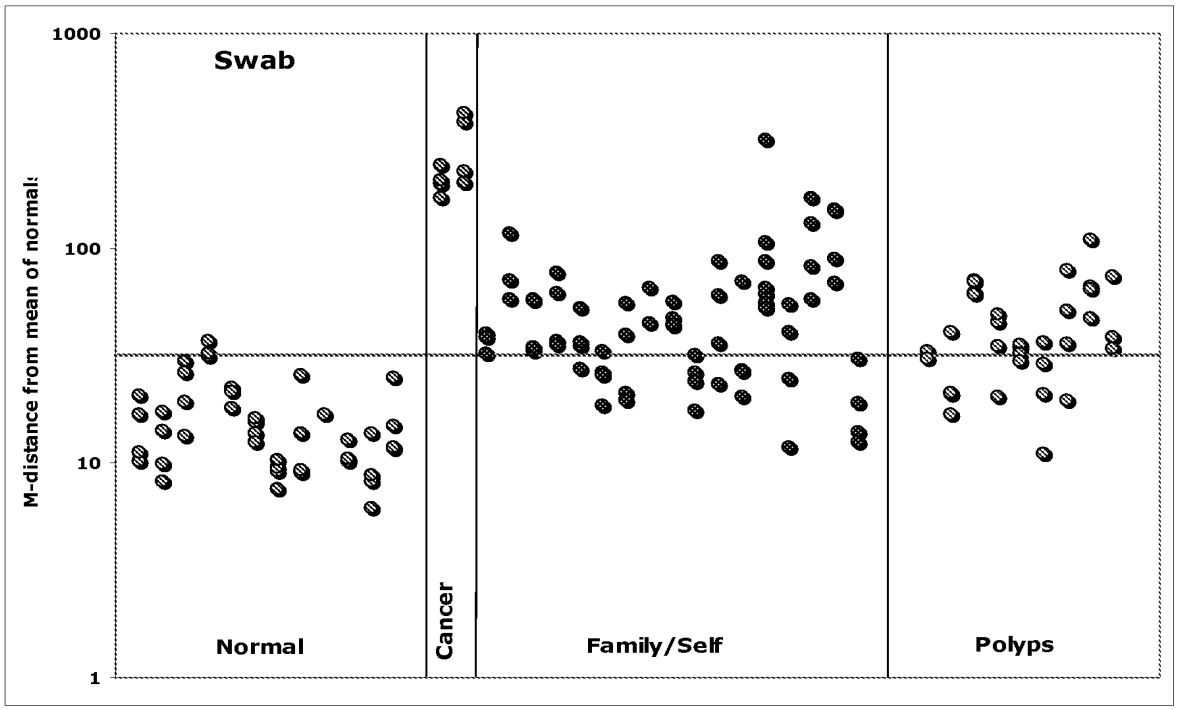



FIGURE 1C

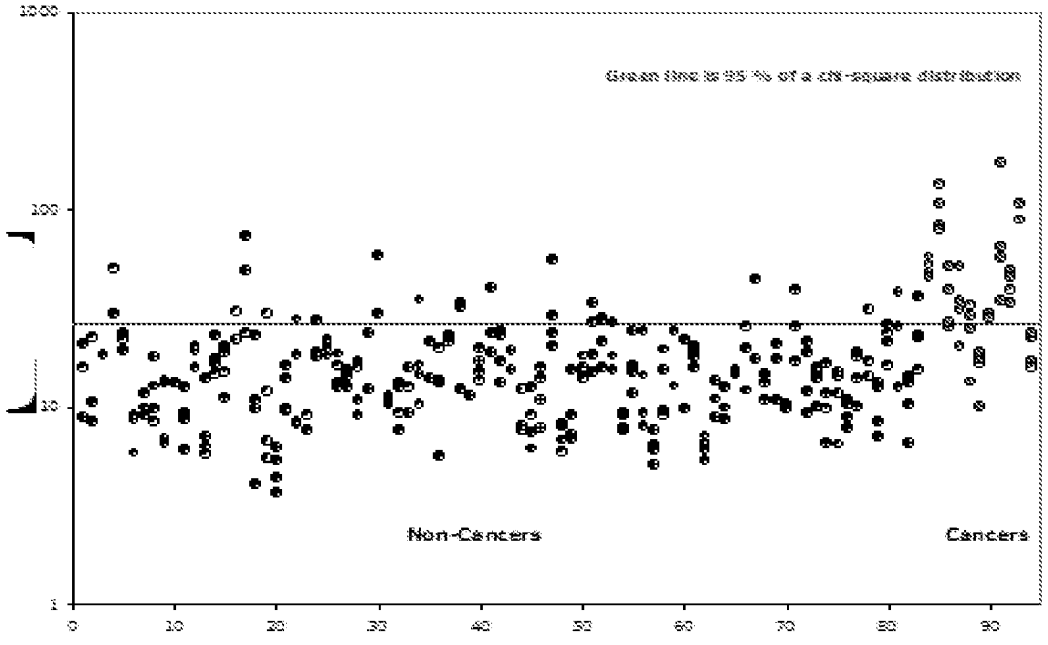



FIGURE 2A

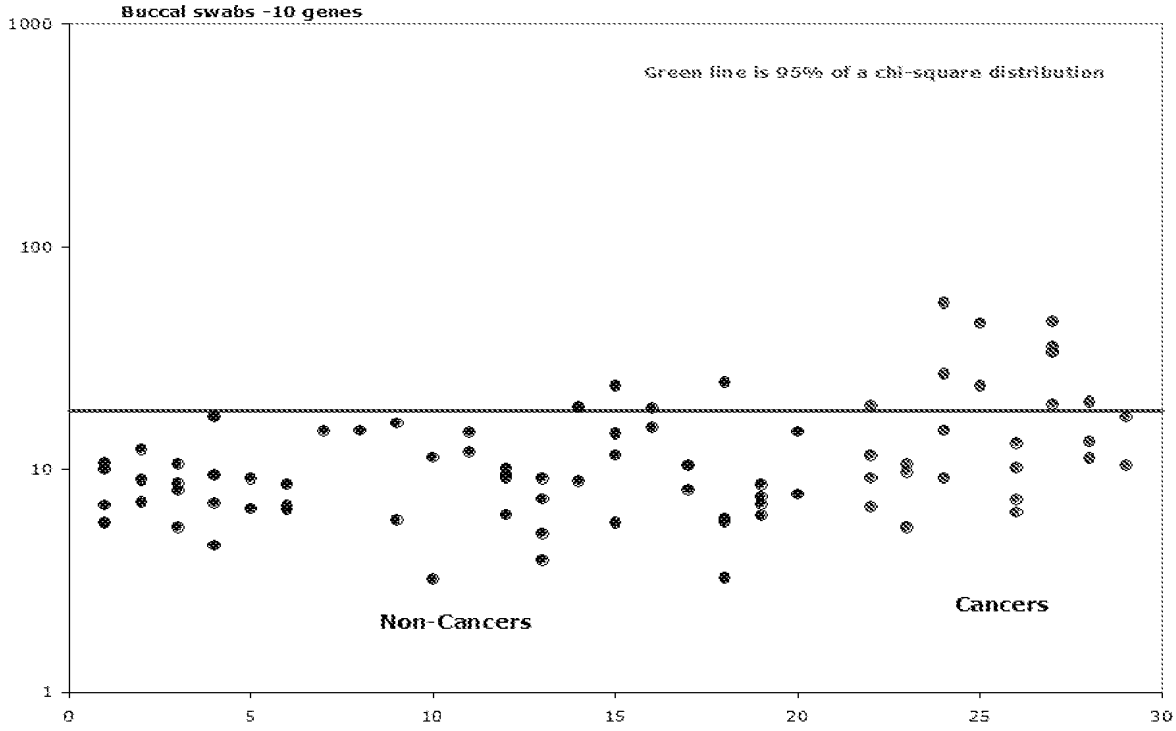

Buccal swabs -10 genes
Green line is 95% of a chi-square distribution
1000
100
10
1
0
5
10
15
20
25
30
Non-Cancers
Cancers

FIGURE 2B